



Short communication

Detection of aflatoxins and zearalenone contamination in wheat derived products

Shahzad Zafar Iqbal^{a,b,*}, Muhammad Rafique Asi^c, S. Jinap^{a,d}, Umer Rashid^e^a Department of Applied Chemistry, Government College University Faisalabad, 38000, Pakistan^b Food Safety Research Centre (FOSREC), Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia^c Food Toxicology Lab, Plant Protection Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan^d Institute of Tropical Agriculture (ITA), Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia^e Institute of Advanced Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:

Received 30 April 2013

Received in revised form

13 June 2013

Accepted 25 June 2013

Keywords:

Aflatoxins

Zearalenone

RP-HPLC

Wheat products

ABSTRACT

In present study the natural occurrence of aflatoxins (AFs) and zearalenone (ZEN) has determined in 147 samples of wheat derived products i.e. spaghetti (25), noodles (34), macaroni (29), lasagne (37), and bucatini (22), collected from major districts of Punjab, Pakistan. The mean of AFs was found 9.12 in spaghetti, 7.35 in noodles, 5.91 in macaroni, 8.39 in lasagne, and 9.61 µg/kg in bucatini and 28, 18, 17, 22 and 27% of samples were found above the European Union's legal limit (i.e. 4 µg/kg) for total AFs, respectively. However, mean value of ZEN was found 7.36 in spaghetti, 6.80 in noodles, 4.98 in macaroni, 6.90 in lasagne, and 8.89 µg/kg in bucatini samples and 16, 15, 21, 19 and 36% of samples were found to be above the recommended limit. The study urged the need to focus more comprehensive survey for these toxins in wheat derived products from Punjab, Pakistan.

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1. Introduction

Food contamination with mycotoxins is a serious issue in developing countries that poses a significant health risks for human as well as for animals (Moss, 1996). AFs represent the most toxic class of mycotoxins, which are mainly produced by species *Aspergillus*, i.e. *Aspergillus flavus* and *Aspergillus parasiticus* and rarely by *Aspergillus nomius* (Iqbal & Asi, 2013). It has been reported that the most toxic class of AFs is aflatoxin B₁ (AFB₁) (Pittet, 1998). Considering the toxicity and carcinogenicity of AFB₁, International Agency for Research on Cancer has placed it as group 1 carcinogen, which mainly affects liver (IARC, 1993, pp. 397–444). ZEN has also common food contaminant, especially in cereals (Jestoi, 2008). It is a non-steroidal estrogenic mycotoxin produced as a secondary metabolite by various *Fusarium* fungi like *Fusarium graminearum* and *Fusarium culmorum* (Bennett & Klich, 2003). It is well known that ZEN is associated with estrogenic effects, including infertility, reduced serum testosterone levels and sperm counts, reduced the incidence of pregnancy, and a change in the progesterone level (Shier, Sier, Xie,

& Mirocha, 2001). International Agency for Research on Cancer has placed it in group 3 carcinogen (IARC, 1993, pp. 397–444).

Pakistan stands 6th in wheat producing countries with production of about 24 million tons in 2010 and this crop is a staple food of this country (Iqbal, Asi, & Jinap, 2013). Due to tropical climatic conditions, wheat is vulnerable for the attack of fungi during pre-harvest or postharvest conditions (Riba, Mokrane, Mathieu, Lebrhi, & Sabaou, 2008). The consumption of wheat derived products (pasta products) is increasing, although it is not a traditional Pakistani cuisine but the consumers are influenced from western food habits. Furthermore, children and younger age consumers like pasta products because of different variation, taste and their ease in cooking. There is very limited data reported for the presence of AFs contamination in wheat products from Pakistan (Lutfullah & Hussain, 2012) and no study was conducted for the presence of ZEN in wheat or pasta products from Pakistan. To minimize the health risks of these mycotoxins in food and food products, European Union (EU) has established permissible limits i.e. 0.1 and 2.0 µg/kg for AFB₁ in cereals products intended for young children and adults, respectively. The limits of 4 µg/kg and 20 µg/kg were established for total AFs and ZEN in processed cereals, respectively (EU Directives, 2006).

Therefore, considering the above circumstances and keeping in view the increase in consumption of pasta products (spaghetti,

* Corresponding author. Food Safety Research Centre (FOSREC), Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia. Tel.: +60 126861121 (cell).

E-mail address: shahzad10542005@yahoo.com (S.Z. Iqbal).

Table 1

The performance parameters for HPLC method for the analysis of aflatoxins and zearalenone.

Mycotoxins	Linearity ($\mu\text{g}/\text{kg}$)	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)	R^2	Precision (%RSD)	
					Repeatability	Reproducibility
AFB ₁	0.1–110	0.04	0.12	0.9989	7	9
AFB ₂	0.5–12	0.06	0.18	0.9974	10	12
AFG ₁	1–100	0.04	0.12	0.9991	8	14
AFG ₂	0.5–12	0.06	0.18	0.9983	8	11
ZEN	0.5–50	0.05	0.15	0.9988	9	13

RSD: relative standard deviation; LOD: limit of detection; LOQ: limit of quantification. Repeatability and reproducibility are given as mean percent RSD.

noodles, macaroni, lasagne, and bucatini); it becomes very important to investigate the natural occurrence of these toxic mycotoxins. The study has focused to investigate the natural occurrence of AFs and ZEN in pasta products from main cities of Punjab, Pakistan and to compare the contamination levels with EU permissible limits.

2. Materials and methods

2.1. Sampling

A total of 147 samples of wheat products, mainly related to pasta i.e. spaghetti (25), noodles (34), macaroni (29), lasagne (37), and bucatini (22) of different brands were purchased randomly from retail outlets, super markets, cafeterias and hotels from main cities of Punjab, Pakistan. The size of sample was more than 0.5 kg. The samples were stored in a freezer at $-4\text{ }^\circ\text{C}$ until further analysis.

2.2. Chemicals

The standards of AFs and ZEN (Sigma–Aldrich, St. Louis, Mo., USA) were provided by Food Toxicology Lab., Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. The immunoaffinity columns (AflaStar & ZearaStar) were purchased from Romers, Stylemaster, USA. Acetonitrile and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA) from Sigma Aldrich (MO, USA). All other chemicals and reagents used were at least of analytical grade.

2.3. Extraction of AFs

The extraction of AFs from wheat derived products was performed according to our previously validated method in our lab by Iqbal et al. (2013).

2.4. Pre-column derivatization

After evaporation 100 ml TFA was added to the residues or AFs standards to derivatize AFB₁ and AFG₁. The samples were placed in the dark place at room temperature for 20 min with caps were tightly on the vials. Then, 0.4 ml of acetonitrile: water (1:9, v/v) mixture was added to the vials. A 20 ml portion of the solution was subjected to LC analysis.

2.5. Extraction of zearalenone

The extraction of ZEN in wheat derived products was performed according to the procedure described by Zaid, Zouaoui, Bacha, and Abid (2012) briefly as, 25 g of each ground sample was extracted with 100 ml mixture of acetonitrile–water (90:10, v/v) by high speed blending for 30 min. Then, the samples were centrifuged for 15 min at 4200 rpm and 20 ml of the filtrate was collected and diluted with 20 ml of distilled water. The diluted extract was cleaned-up through immunoaffinity column at a flow rate of 1–2 drops/s. The column was washed twice with 5 ml of distilled water and ZEN was then slowly eluted from the column with 3 ml of HPLC grade methanol. The eluted sample was evaporated to dryness at $70\text{ }^\circ\text{C}$ and the residue was reconstituted in 250 μl of mobile phase prior to the injection to HPLC.

2.6. HPLC conditions

The HPLC used was a Shimadzu (LC-10A series, Kyoto, Japan) equipped with fluorescence detector (RF-530). The chromatographic column was Supelco C18 Column ($4.6 \times 250\text{ mm}$, $5\text{ }\mu\text{m}$; Discovery, HS, Bellefonte, USA). The mobile phase used for AFs analysis was acetonitrile–methanol–water (20:20:60, v/v/v) with a flow rate of 1 ml/min. The mobile phase used for ZEN analysis was acetonitrile–water–methanol (48:50:2 v/v/v) with a flow rate of 1.0 ml/min. The injection volume was 20 μl . Excitation and emission wavelengths were set at 360 and 440 nm for AFs and 274 and 450 nm for ZEN, respectively.

Table 2

Recoveries with fortified level of aflatoxins and zearalenone in wheat products.

Mycotoxins	Spiked level ($\mu\text{g}/\text{kg}$)	Spaghetti			Noodles			Macaroni			Lasagne			Bucatini		
		Mean ($\mu\text{g}/\text{kg}$)	Recovery (%)	RSD (%)	Mean ($\mu\text{g}/\text{kg}$)	Recovery (%)	RSD (%)	Mean ($\mu\text{g}/\text{kg}$)	Recovery (%)	RSD (%)	Mean ($\mu\text{g}/\text{kg}$)	Recovery (%)	RSD (%)	Mean ($\mu\text{g}/\text{kg}$)	Recovery (%)	RSD (%)
AFB ₁	2	1.72	86	10	1.74	87	9	1.64	82	10	1.69	84	8	1.62	81	9
	4	3.49	87	10	3.48	87	10	3.59	90	11	3.56	89	10	3.49	87	12
	6	5.47	91	5	5.49	92	5	5.60	93	6	5.57	93	6	5.65	94	8
AFG ₁	2	1.71	85	11	1.68	84	9	1.84	92	12	1.69	84	9	1.63	82	14
	4	3.49	87	10	3.51	88	10	3.35	84	9	3.51	88	10	3.71	92	9
	6	5.50	92	5	5.52	92	5	5.47	91	5	5.51	92	5	5.47	91	11
AFB ₂	2	1.69	84	7	1.71	86	9	1.79	90	8	1.66	83	19	1.48	74	9
	4	3.51	88	9	3.53	88	9	3.51	88	9	3.48	87	10	3.69	92	7
	6	5.52	92	5	5.54	92	5	5.75	95	5	5.54	92	5	5.75	95	12
AFG ₂	2	1.58	78	16	1.64	82	10	1.71	85	16	1.64	82	9	1.53	77	11
	4	3.46	86	12	3.50	88	12	3.59	90	11	3.43	86	13	3.68	92	9
	6	5.52	92	6	5.55	92	6	5.48	91	7	5.55	92	5	5.72	95	17
Zearalenone	2	1.64	82	19	1.68	84	15	1.71	85	15	1.69	84	16	1.47	74	12
	4	3.68	92	5	3.65	91	4	3.54	88	6	3.64	91	6	3.55	88	14
	6	5.35	89	4	5.52	92	6	5.38	89	4	5.42	90	5	5.49	92	16

Mean is the average of 5 replicates of each concentration of fortified level.

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